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PPM1H Purification from E. coli

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is used to purify full length PPM1H enzyme in good yield and purity after expression in bacteria. We generally obtain 2mg from 4L bacterial culture and express the protein at 18°C to avoid aggregation due to overexpression.

Materials

Lysis Buffer:
- 50mM HEPES pH 8
- 500mM NaCl
- 5mM $MgCl_2$
- 0.5 mM TCEP
- EDTA free Protease tablet (1/50mL)
- 10% glycerol

Wash Buffer:
- 50mM HEPES pH 8
- 500mM NaCl
- 5mM MgCl2
- 20mM imidazole
- 0.5 mM TCEP
- 10% glycerol

Elution Buffer:
- 50mM HEPES pH 8
- 500mM NaCl
- 5mM $MgCl_2$
- 500mM imidazole
- 0.5 mM TCEP
- 10% glycerol

Size Exclusion/Dialysis Buffer:
- 50mM HEPES pH 8
- 150mM NaCl
- 5mM $MgCl_2$
- 0.5 mM TCEP
- 10% glycerol

LB agar plates containing 100µg/ml carbenicillen and 34µg/ml chloramphenicol
Per flask, 2ml each of 1000X carbenicillin (100mg/ml in H_2O) and chloramphenicol (34mg/ml in EtOH)
Roche EDTA-free Protease inhibitor tablets
Avestin Emulsiflex unit for cell breakage
SUMO protease (Ulp1)

Troubleshooting

Day 1: Prepare a Starter Culture and Sterilize media

1 Pick a fresh colony from an LB agar plate to inoculate 50 mL LB broth. Grow Overnight with rotation at 37 °C

2 Autoclave 2 × 6L flasks, each containing 2 L LB broth. Store at 37 °C for inoculation the following day

Day 2: Inoculation and Induction

3 Inoculate each 6L flask with 20 mL of starter culture

4 Add 2 mL, 1000X Carbenicillin and 2 mL, 1000X chloramphenicol to flask and rotate at 37 °C until OD_{600} 0.5-0.6 is reached

4.1 Take an initial OD measurement 1hr post inoculation

4.2 Doubling time is 20-30min. Calculate how long to reach 0.6 (usually 2.5 hr)

5 Optional: use remaining O/N culture to make additional bacterial glycerol stocks

5.1 Glycerol stocks are made using 500 µL of bacterial culture and 500 µL of 50% Glycerol

5.2 Transfer to -80 °C immediately

6 Once OD is reached, transfer flasks to 18 °C controlled temperature shaker and equilibrate temperature for 10-20min

7 Add IPTG to [M] 0.3 millimolar (mM)

8 Incubate with rotation overnight (180 RPM) at 18 °C

Day 3: Purification Protocol

9 Resuspension and Lysis

9.1 Transfer cultures to 1L centrifuge bottles

9.2 Pellet bacteria by spinning at 4000 RPM in a Sorvall centrifuge for 00:20:00 at 4 °C ; discard supernatant 20m

9.3 Add 1 Roche protease inhibitor tablet to 50mL of lysis buffer, rotate ~10 min at 4 °C to dissolve

9.4 Set up a ~200mL beaker with a magnetic stir bar at 4 °C

9.5 Resuspend pelleted bacteria on ice or in cold room using ~ 10 mL lysis buffer per bottle

9.6 Vortex the bottles to resuspend the pellet

9.7 Transfer slurry to 200ml beaker with stir bar

9.8 Pass 20 mL lysis buffer through the Emulsiflex at 0-30K PSI to equilibrate machine

9.9 Pass bacterial slurry through Emulsiflex once at 40-60K PSI to lyse cells. Collect into Oakridge tube(s)

9.10 If the slurry resists pipetting up and down, add additional lysis buffer (no more than 20 mL more)

9.11 Balance tubes

9.12 Centrifuge at 40,000 RPM for 45min in a 45Ti rotor at 4 °C to pellet cell debris

9.13 Collect supernatant and pass through a 500ml, 0.45µm Millipore filter under vacuum. Transfer the clarified supernatant into a 50ml glass beaker

10 HiTrap Talon (Cobalt) Affinity Purification using FPLC

10.1 Wash a 1mL HiTrap Talon column with 10 column volumes (CV) of distilled degassed H_2O

10.2 Equilibrate column with 10CV of lysis buffer

10.3 Pass the lysate through the column. Collect the flow through

10.4 Wash the column with 30CV of wash buffer. Collect the wash fractions

10.5 Elute using an imidazole gradient of 100mM-500mM. Collect the elution fractions and analyze by SDS PAGE

10.6 Pool the eluted fractions containing the desired protein and dialyze overnight into dialysis buffer in the presence of SUMO protease (Ulp1) (100ng/1mg of protein). We usually dialyze ~6mL

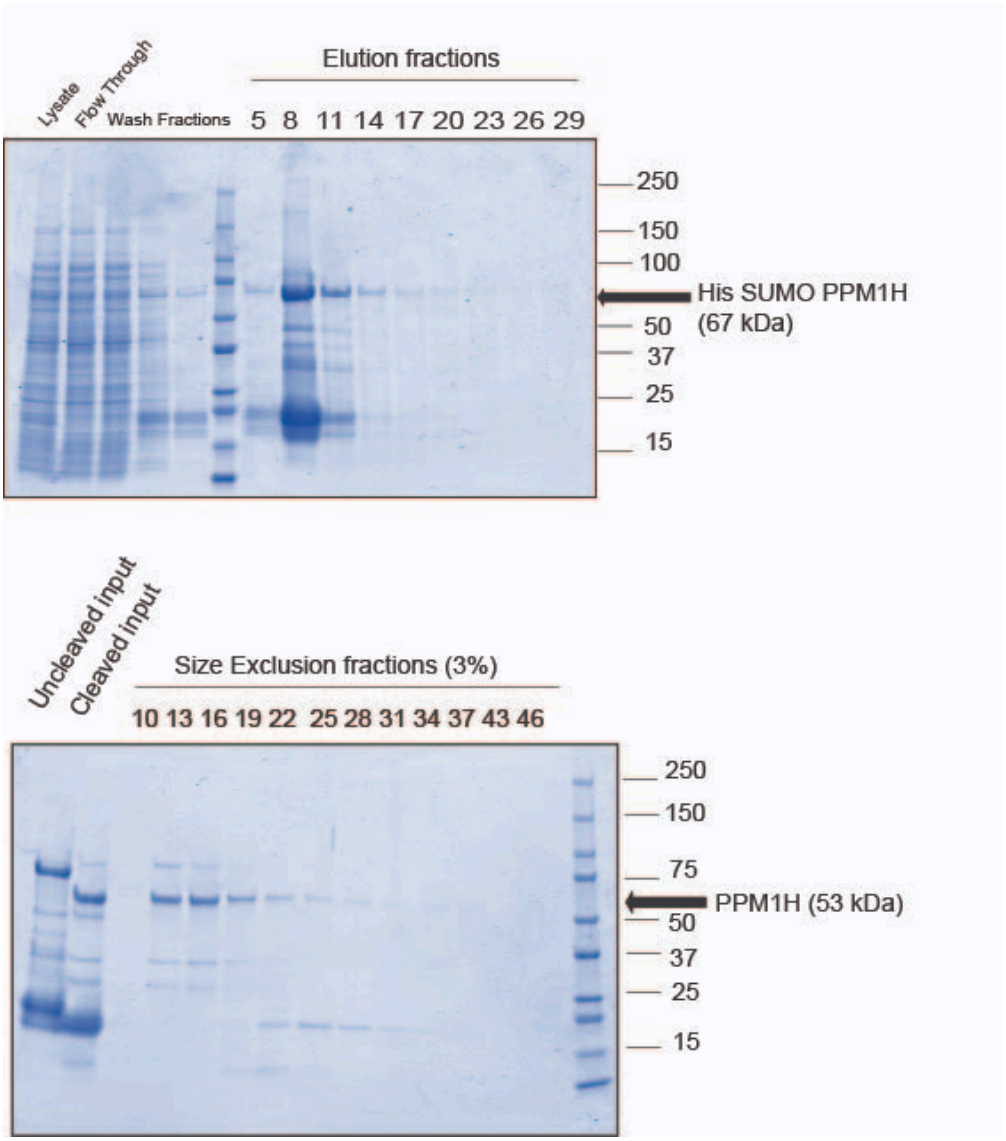
10.7 Collect the dialyzed protein the next day and concentrate it by microfiltration on an Centricon 10K cutoff filter before resolving the protein on a S200 24 ml column fitted in series with a 1ml HiTrap Talon column to remove uncleaved His SUMO protein and the His-tagged SUMO protease

10.8 Collect 4-5, 4ml fractions. Analyze by SDS-PAGE and pool the fractions containing the desired protein

10.9 Concentrate the protein by Centricon filtration if needed

10.10 Snap freeze in liquid nitrogen and store at -80 °C

11 Shown below are SDS-PAGE gels of the initial Cobalt column and gel filtration fractions after His-SUMO tag removal



SDS-PAGE of column fractions from this protocol; yield is ~2mg/4L culture

Protocol references

Yeshaw WM, Adhikari A, Chiang CY, Dhekne HS, Wawro PS, Pfeffer SR. Localization of PPM1H phosphatase tunes Parkinson's disease-linked LRRK2 kinase-mediated Rab GTPase phosphorylation and ciliogenesis. Proc Natl Acad Sci U S A. 2023 Oct 31;120(44):e2315171120. doi: 10.1073/pnas.2315171120.
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